Chemical Kinetics: Catalysis

- Enzyme Mechanisms
  - The nitty-gritty of enzyme/substrate dependencies
  - Michaelis-Menten Mechanism
    - k_{cat} and K_M, turn-over number
    - Lineweaver-Burke plots
  - Bisubstrate Reactions
  - Inhibition

A standard textbook on Enzyme Kinetics (Irving Segal) lists over 50 differentiable mechanisms for enzymatic function:

$$E + S \underset{k_{-1}}{\overset{k_1}{\longleftrightarrow}} ES \xrightarrow{k_{cat}} E + P$$ Michaelis-Menten

$$\begin{array}{l} E + S \underset{k_{-1}}{\overset{k_1}{\longleftrightarrow}} ES \xrightarrow{k_{cat}} E + P \\ + \\ I \\ \updownarrow \\ EI \end{array}$$ Competetive Inhibition

$$\begin{array}{l} E + S_1 \underset{k_{-1}}{\overset{k_1}{\longleftrightarrow}} ES_1 \xrightarrow{k_{cat}} E' + P_1 \\ + \\ S_2 \\ \updownarrow \\ E'S_2 \xrightarrow{k_{cat}} E + P_2 \end{array}$$ Iso-Ordered Ping-Pong

For all these reaction types there are a fairly algorithmic way of solving for their rate laws. In all cases this involves:

1) Writing them down as elementary reaction steps
2) Making approximations:
    a) stationary state approximation
    b) rapid equilibrium approximation
    c) conserved total enzyme
    d) large but not too large concentration of S's
    e) small but not too small concentration of E
    f) we are interested in only initial rates

$$
\begin{array}{ccccccccc}
E & + & S & \underset{k_{-1}}{\overset{k_1}{\longleftrightarrow}} & ES & \xrightarrow{k_{cat}} & E & + & P \\
+ & & & & & & & & \\
I & & & & \updownarrow & & & & \\
\updownarrow & & & & & & & & \\
EI & + & S & \longleftrightarrow & EIS & & & &
\end{array}
$$

$$E + S \underset{k_{-1}}{\overset{k_1}{\longleftrightarrow}} ES \xrightarrow{k_{cat}} E + P$$ Michaelis-Menten

We have seen the example of the Michaelis-Menten mechanisms many times now.

They solve via the stationary-state approximation:

$$v_0 = k_{cat}[ES]$$

$$E_t = [E] + [ES]$$

$$\frac{d[ES]}{dt} = k_1[E][S] - (k_{-1} + k_{cat})[ES] = 0$$

Enzymes are present at about 10^{-8}-10^{-10}M.

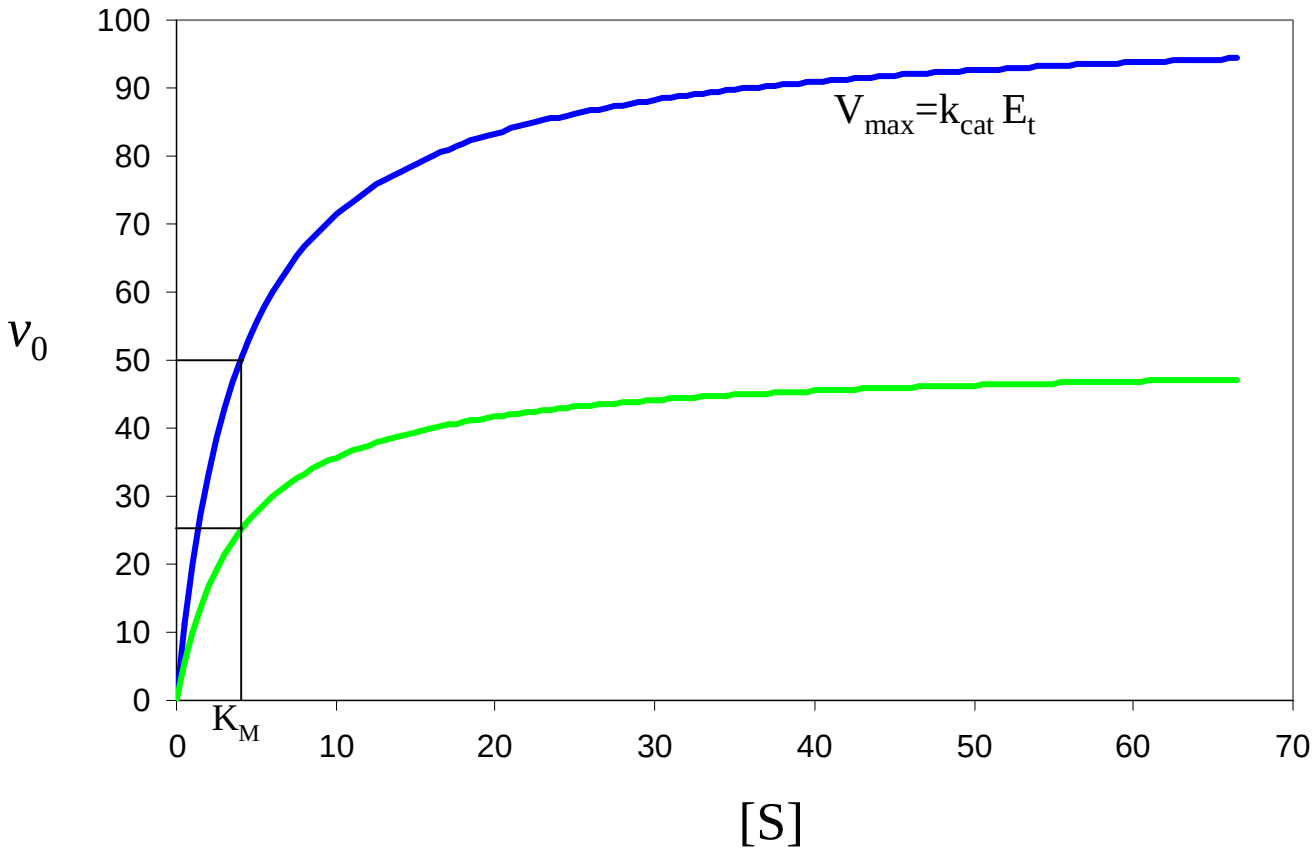
Substrates at more like micromolar to millimolar

$$k_1(E_t - [ES])[S] - (k_{-1} + k_{cat})[ES] = 0$$

$$[ES] = \frac{E_t S}{\frac{k_{-1} + k_{cat}}{k_1} + S} = \frac{E_t S}{K_M + S}$$

The rate of product production for a Michaelis-Menten enzyme is thus

$$v_0 = k_{cat}[ES] = k_{cat}\frac{E_t S}{K_M + S}$$



We generally talk about initial rates because otherwise, as P gets large, there must be a back reaction (remember thermodynamics!)

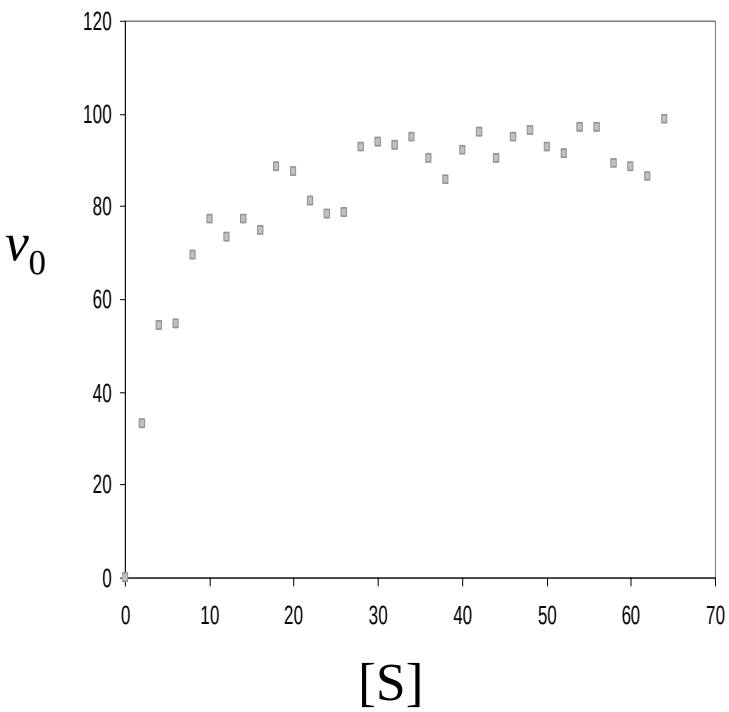
$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \underset{k_{rev}}{\overset{k_{cat}}{\rightleftarrows}} E + P$$

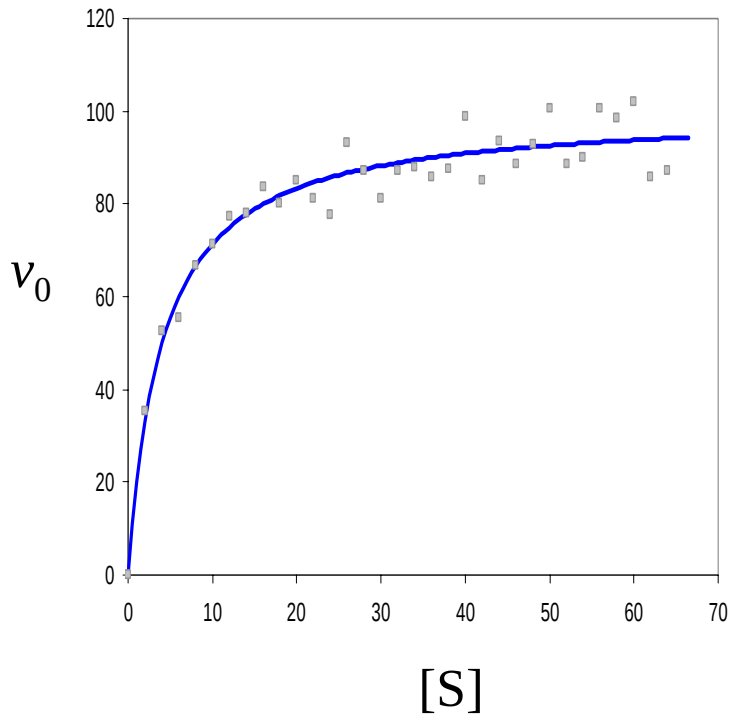
So back to the Michaelis-Menten Equation

$$v_0 = k_{cat}[ES] = \frac{k_{cat}E_tS}{\frac{k_{-1}+k_{cat}}{k_1}+S} = \frac{k_{cat}E_tS}{K_M+S}$$

Notice that if $k_{-1} >> k_{cat}$ then the Michaelis constant is the dissociation constant! This *is* the rapid equilibrium assumption!

Now when we are doing real experiments our velocity curves don't look so good!





It's difficult to fit the line through the data with standard tools….much better to do a linear regression!

Lineweaver and Burk came up with the following simple transform:

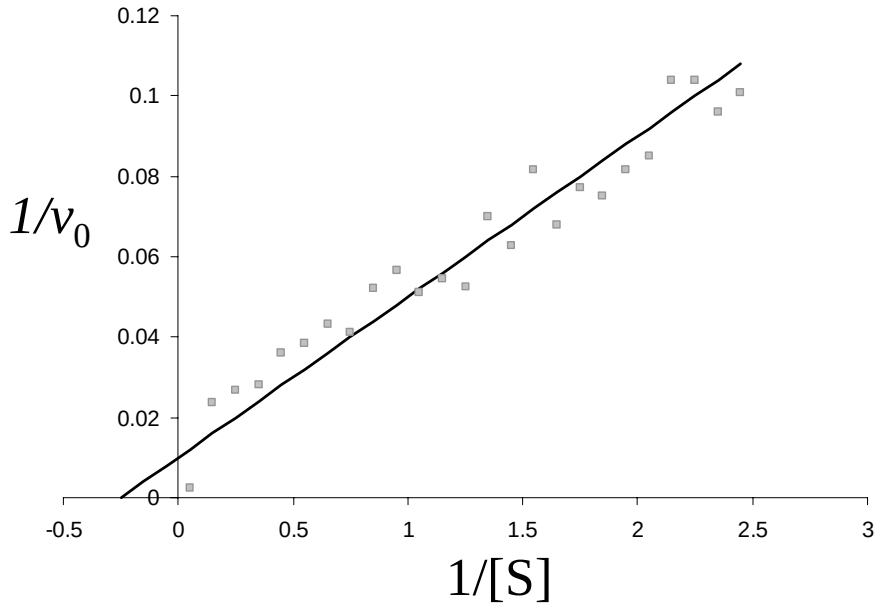
$$v_0 = k_{cat}[ES] = k_{cat}\frac{E_t S}{K_M + S}$$

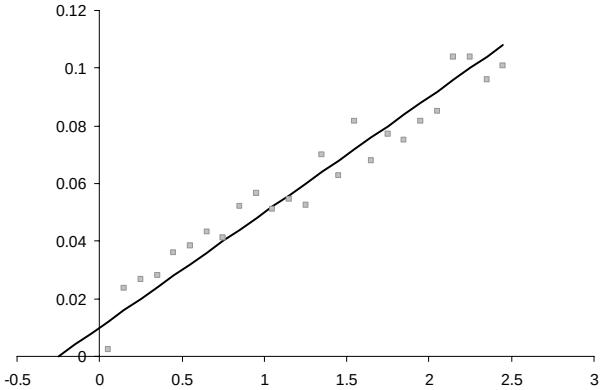
$$\frac{1}{v_0} = \frac{1}{k_{cat}}\frac{K_M + S}{E_t S} = \frac{1}{V_{\max}}\left(1 + \frac{K_M}{S}\right)$$

So plotting $1/v_0$ versus 1/[S] gives a linear plot with

y-intercept= $1/V_{max}$
slope= K_m/V_{max}

x-intercept= $-1/K_M$





y-intercept= $1/V_{max}$
slope= K_m/V_{max}
x-intercept= $-1/K_M$

So Lineweaver-Burk plots are linear for Michaelis-Menten Enzymes.

How do they look for more complicated cases?

Note that they give measure of K_m and V_{max}.

But V_{max} is a non-specific measure unless you have absolutely pure enzyme in your prep and you know its molecular weight!

Only k_{cat} is a measure of the intrinsic activity of the enzyme!

$$E + S \underset{k_{-1}}{\overset{k_1}{\longleftrightarrow}} ES \xrightarrow{k_{cat}} E + P$$

These rates are also called ‘turn-over’ numbers since they are a measure of how many molecules the enzyme can process per second working at maximum rate!

$$E + S \underset{k_{-1}}{\overset{k_1}{\longleftrightarrow}} ES \xrightarrow{k_{cat}} E + P$$

In general, V_{max} ranges from between 100-10,000,000 per second.

However, the overall rate of the production of product from substrate is lower due to the “Collision probability” term.

$$v_0 = k_{cat}[ES] = k_{cat}\frac{E_t S}{K_M + S}$$

When this term is dominates by k_1 then it is diffusion controlled.

(Sometime, V_{max} is estimated to be larger than can be explain by collision.)

Enzymes can be affected by binding of non-substrate molecules.
Inhibition can occur through numerous routes.

$$E + S \underset{k_{-1}}{\overset{k_1}{\longleftrightarrow}} ES \xrightarrow{k_{cat}} E + P$$

$$+$$
$$I$$
$$\updownarrow$$
$$EI$$

Competitive Inhibition

$$E + S \underset{k_{-1}}{\overset{k_1}{\longleftrightarrow}} ES \xrightarrow{k_{cat}} E + P$$

$$+ \qquad\qquad \updownarrow$$
$$I \qquad\qquad \updownarrow$$
$$EI + S \longleftrightarrow EIS$$

Non-competitive Inhibition

And don’t forget cooperative/allosteric models.

The effect of a competitive inhibitor is to remove some enzyme from the pool available to interact with S.

$$E + S \underset{k_{-1}}{\overset{k_1}{\leftrightarrow}} ES \xrightarrow{k_{cat}} E + P$$

$$+ I \updownarrow EI$$

Competitive Inhibition

$$K_S = [E][S]/[ES]$$

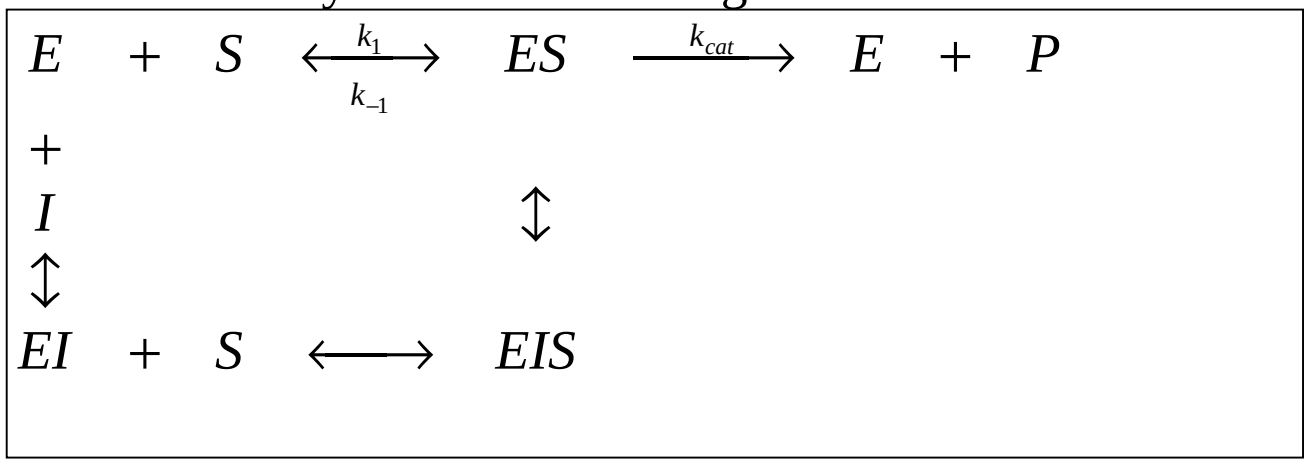
$$K_I = [E][I]/[EI]$$

$$E_t = [E] + [EI] + [ES] = \frac{K_S}{[S]}[ES] + \frac{[I]}{K_I}\frac{K_S}{[S]}[ES] + [ES]$$

$$[ES] = \frac{E_t[S]}{K_S + \frac{K_s}{K_I}[I] + [S]} = \frac{E_t[S]}{K_S(1 + \frac{[I]}{K_I}) + [S]}$$

The apparent effect is to lower the apparent binding constant of the enzyme for the substrate.

On the other hand for a system like this we get:



$$K_S = [E][S]/[ES]$$

$$K_I = [E][I]/[EI]$$

$$E_t = [E]+[EI]+[EIS]+[ES] = \frac{K_S}{[S]}[ES]+\frac{[I]}{K_I}\frac{K_S}{[S]}[ES]+\frac{[I]}{K_I}[ES]+[ES]$$

$$E_t = [ES]\left(\frac{K_S}{[S]}\left(1+\frac{I}{K_I}\right)+\left(\frac{I}{K_I}+1\right)\right)$$

$$[ES] = \frac{[S]}{K_S+[S]}\frac{E_t}{\left(1+\frac{I}{K_I}\right)}$$

Which shows that the net effect of the inhibitor is to decrease the apparent V_{max} of the enzyme.

$$[ES] = \frac{E_t[S]}{K_S + [S]}$$

Uninhibited

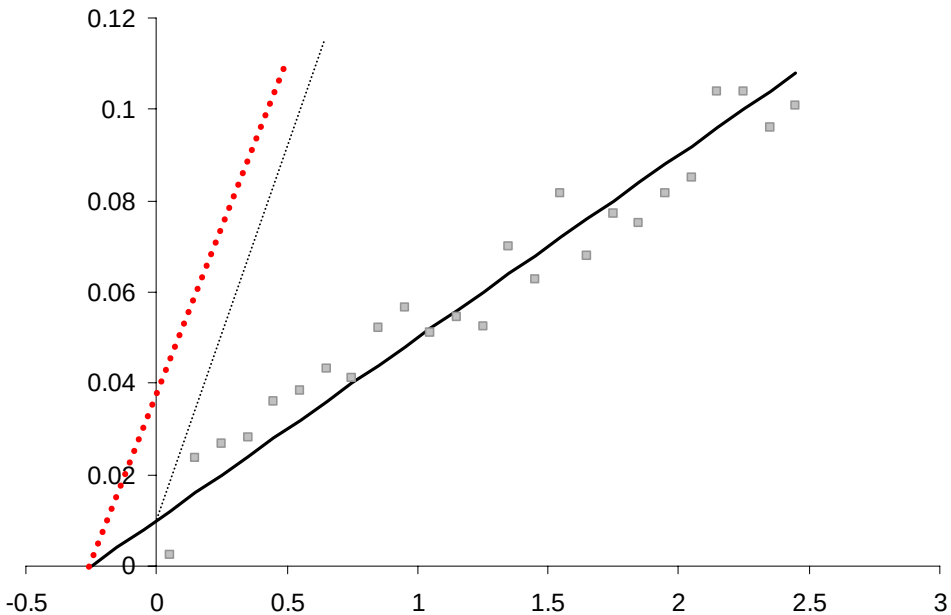
$$[ES] = \frac{E_t[S]}{K_S(1 + \frac{[I]}{K_I}) + [S]}$$

Competitive

$$[ES] = \frac{[S]}{K_S + [S]} \frac{E_t}{\left(1 + \frac{I}{K_I}\right)}$$

Non-Competitive

What will be the effects on a Linweaver-Burk plot!



y-intercept= $1/V_{max}$
slope= K_m/V_{max}
x-intercept= $-1/K_M$

What happens in more complex mechanisms?

$$E + S_1 \underset{k_{-1}}{\overset{k_1}{\longleftrightarrow}} ES_1 \underset{k_{-2}}{\overset{k_2}{\longleftrightarrow}} EP \xrightarrow{k_{cat}} E + P$$

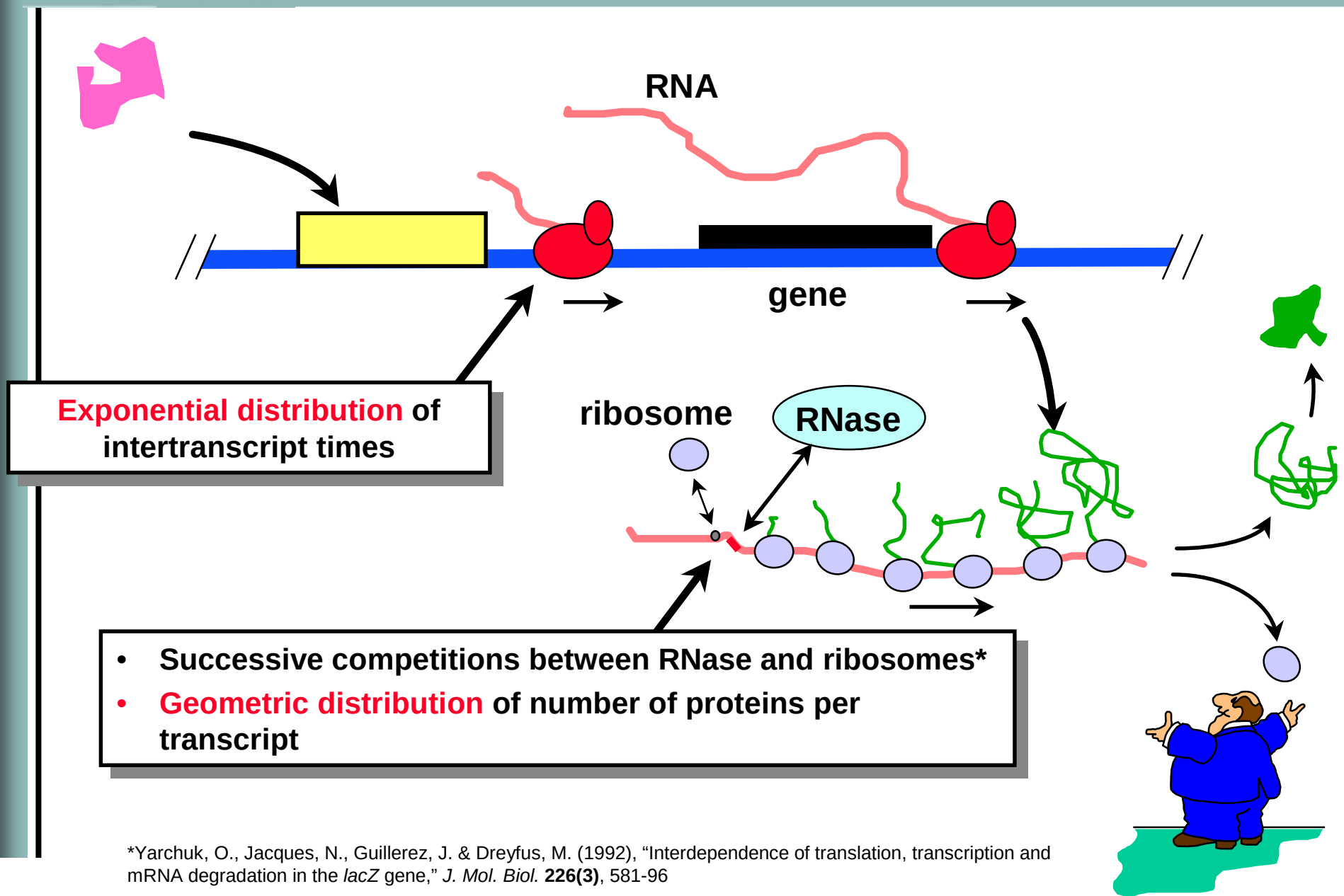
$$\begin{array}{l} E + S_1 \underset{k_{-1}}{\overset{k_1}{\longleftrightarrow}} ES_1 \xrightarrow{k_{cat}} E' + P_1 \\ \qquad\qquad\qquad\qquad\quad + \\ \qquad\qquad\qquad\qquad\quad S_2 \\ \qquad\qquad\qquad\qquad\quad \updownarrow \\ \qquad\qquad\qquad\qquad\quad E'S_2 \xrightarrow{k_{cat}} E + P_2 \end{array}$$

This last reaction mechanism, for example, is what happens with superoxide dismutase.

$$O_2^- + E \longleftrightarrow E \bullet O_2^- \longrightarrow E' + O_2$$

$$O_2^- + E' \longleftrightarrow E' \bullet O_2^- \longrightarrow E + H_2O_2$$

Stochastic Mechanisms in Gene Expression



*Yarchuk, O., Jacques, N., Guillerez, J. & Dreyfus, M. (1992), "Interdependence of translation, transcription and mRNA degradation in the *lacZ* gene," *J. Mol. Biol.* **226(3)**, 581-96

Some Stochastic Cellular Phenomena

- Lineage commitment in human hemopoiesis

- Random, bimodal eukaryotic gene transcription in
  - Activated T cells
  - Steroid hormone activation of mouse mammary tumor virus
  - HIV-1 virus

- Clonal variation in:
  - Bacterial chemotactic responses
  - Cell cycle timing

- *E. coli* type-1 pili expression
  - Enhances virulence

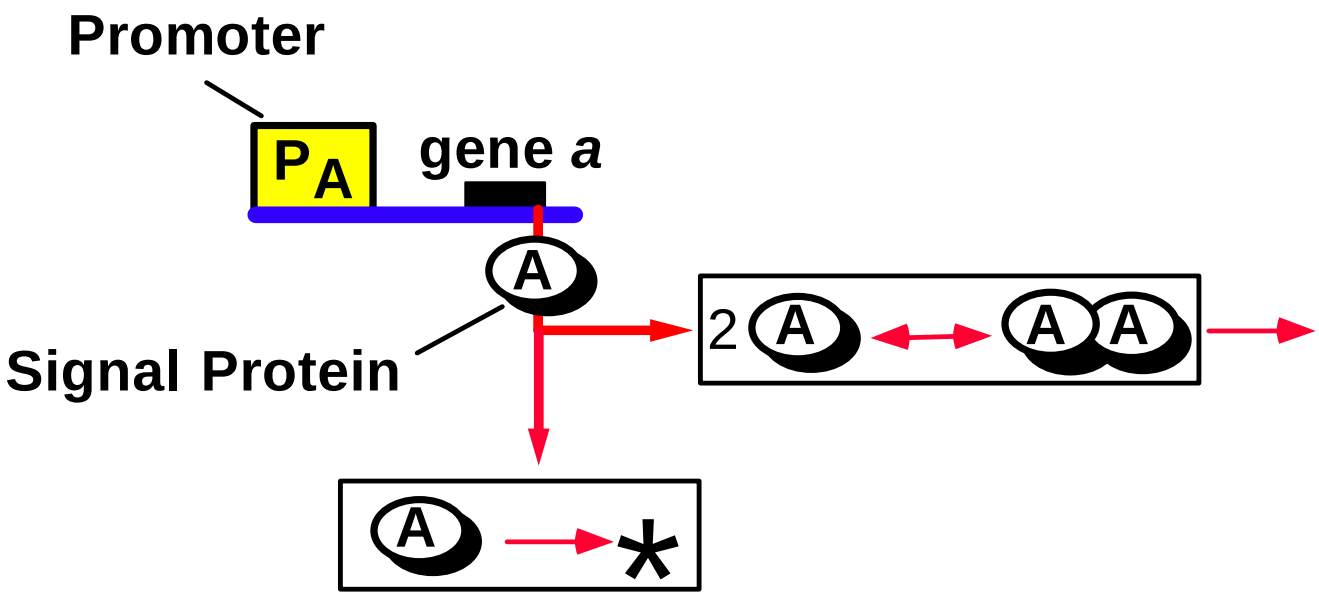
- Changing cell surface protein expression
  - For immune response avoidance

- Bacteriophage λ lysis/lysogeny decision

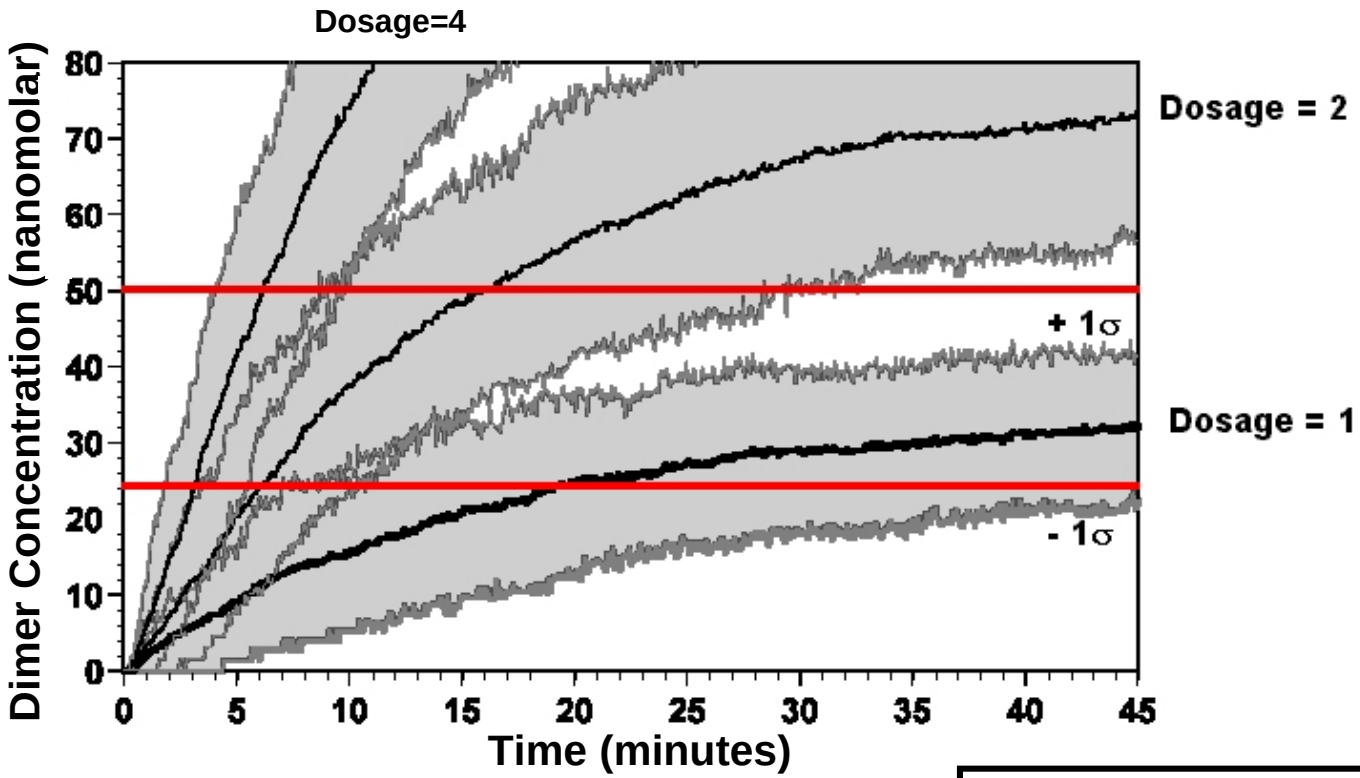
Where Noise Comes From

- Random environmental influences
- Mutations
- Asymmetric partitioning at cell division
- Stochastic mechanisms in gene expression
  - Stochastic timing of gene expression
  - Random variation in time for signal propagation
  - Random variation total protein production

A simple example



Time to Effectivity



Timing uncertainty reduced by:

- **Higher gene dosage**
- **Strong promoter**
- **Multiple promoters**
- **Lower effectivity threshold**
- **Slower cell growth**